SUBMISSION OF PROPOSAL FOR TOPICAPPROVAL IN THE FACULTY OF SCIENCE AND TECHNOLOGY

PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE Ph.D. DEGREE IN THE SUBJECT OF PHARMACEUTICS

Title of the topic	:	Design and Characterization of site- specific drug delivery for breast cancer.
Name of the student	:	Mohite Priyanka Ankush Aparna
Basic Qualification	:	M. Pharmacy
In the case of Ph.D. student Year of Passing of PET/NET/SET/M.Phil /GPAT/GATE (Teaching Experience)	:	2017
Name of the Research Supervisor/Guide	:	Dr. Nalini Satish Kurup
Date of Superannuation/Retirement of the Research Supervisor/Guide	:	31st January 2036
Name of the Research Centre	:	Principal K.M.Kundnani College of Pharmacy,Colaba
Signature of the Student	:	
Signature of the Research Supervisor/Guide	:	

Date: 02/03/2023

Seal of College/ Department

Signature of Head/Director/Principal Research Centre

INDEX

Sr. No.	Contents	Page No.
1	Title	1
2	Aim of study	1
3	Objectives of Study	1
4	Study Background (Literature Review)and significance of study	1-3
5	National Status	3-5
6	International Status	5-8
7	Study Hypotheses	8
8	Study Rationale	8
9	Research Methodology	8-13
10	Statistical Plan	13-14
11	Limitations of the Study	14
12	Expected Outcomes of Study	14
13	Select Bibliography	14-17

1 **Title:** Design and Characterization of site- specific drug delivery for breast cancer.

2 **Aim of study:** Formulation development and characterization of site- specific drug delivery system.

3 **Objectives of Study:**

- To develop a novel formulation that provides site- specific release of drug through novel drug delivery in the form of Microneedle/ Microsponges.
- To develop and validate analytical method for estimation of drug content.
- To study the effect of various polymers as well as different formulation parameters on percent practical yield, percent drug entrapment efficiency, percent drug content and particle size of formulation.
- To optimize the formulation by QbD with the help of DoE software.
- To study drug release by *ex vivo* and *in vitro* methods.
- To perform cell line study to estimate anti-cancer activity of optimized formulation.
- To carry out stability studies on optimized formulation as per conditions mentioned in ICH guidelines.

4 Study Background (Literature Review) and significance of study:

A large number of cases every year report uncontrolled cell growth and are diagnosed with cancer. Among females, breast cancer is the most common type of malignancy and is one of the major causes of their death[1]. The number of breast cancer cases is still increasing every year so; the disease has become a chief concern for health sciences. Development in genomics, proteomics and biomedical research has enabled scientists to unveil the receptors, mediators and signaling pathways involved in breast cancer pathogenesis. Breast cancer is mainly divided in four major molecular classes depending on the expression of hormone receptor (HR) and human epidermal growth factor receptor (HER) i) luminal A (HR+/HER2−); ii) luminal B (HR+/HER2+); iii) HER2+and (iv) triple-negative (TNBC). 60 to 80 % of cases represent the luminal type of breast cancer which over expresses hormone receptors, namely estrogen receptor and progesterone receptor[2].

Hormone-dependent breast cancer can be successfully treated by either blocking the oestrogen receptor (ER), as with tamoxifen, or reducing the production of oestrogen, as with

aromatase inhibitors (AIs) [3]. Third-generation AIs can very efficiently suppress circulating oestrogen levels in postmenopausal women. Among these third-generation AIs, two classes of products interfering with the enzyme aromatase can be distinguished. Anastrozole and letrozole are the representatives of the nonsteroidal agents, whereas exemestane is the single component belonging to the steroidal inhibitors [4].

Breast cancer cell growth may be estrogen-dependent. Aromatase (exemestane) is the principal enzyme that converts androgens to estrogens both in pre- and postmenopausal women[5]. While the main source of estrogen (primarily estradiol) is the ovary in premenopausal women, the principal source of circulating estrogens in postmenopausal women is from conversion of adrenal and ovarian androgens (androstenedione and testosterone) to estrogens (estrone and estradiol) by the aromatase enzyme in peripheral tissues. Estrogen deprivation through aromatase inhibition is an effective and selective treatment for some postmenopausal patients with hormone-dependent breast cancer. Exemestane is an irreversible, steroidal aromatase inactivator, structurally related to the natural substrate androstenedione. It irreversibly binds to the active site causing permanent inhibition necessitating de novo synthesis to restore enzymatic function. Exemestane significantly lowers circulating estrogen concentrations in postmenopausal women, but has no detectable effect on the adrenal biosynthesis of corticosteroids or aldosterone. This reduction in serum and tumor concentrations of estrogen delays tumor growth and disease progression. Exemestane has no effect on other enzymes involved in the steroidogenic pathway up to a concentration at least 600 times higher than that inhibiting the aromatase enzyme[6].

Phytoestrogens, plant-derived polyphenols that structurally and functionally mimic 17β-estradiol, the mammalian estrogen hormone, are known to modulate multiple molecular targets in breast cancer cells[7]. The structural and chemical similarities to estradiol enable phytoestrogens to exert estrogenic or antiestrogenic activities by binding to the estrogen receptors[8].The properties of these compounds are employed for their usage in different purposes such as prevention of cancer, prevention of osteoporosis, improving post-menopausal health condition; etc phytoestrogens can synergize the anti-cancer efficacy of exemestane[9].

Exemestane is available in the market under the trade name "Aromasin" with a dose of 25mg. However, it is reported that only 42% of the dose is absorbed in systemic circulation after its oral administration. Though efficacious, Exemestane shows troublesome pharmacokinetic properties as it belongs to BCS class IV; representing its low solubility and low permeability.Drug undergoes high first-pass metabolism. Exemestane tablets have some undesired effects; including osteoporosis. One method to minimize this is to deliver exemestane as site-specific delivery[10].

5 National Status

Breast cancer is one of the epidemic threats among different cancers in women and technologically advanced approaches have been attempted to overcome breast cancer. Despite conventional therapies being effective, there are several shortcomings, such as undesirable side effects, problems in the bioavailability of some drugs, due to which several advancements have been endeavoured for curing breast cancer. Nano-based advanced drug delivery systems possess tumor targeting ability, reduced side effects, high bioavailability, and controlled release of a drug, and thus, became a research hotspot for breast cancer too. Apart from the nano-based delivery system, micro-based delivery system, transdermal patches, microneedles have similarly been well-thought-out strategies. To enhance targeting, some tactics such as surface ligand attachment and use of stimuli-responsive materials have shown promises.

Mh Kanani et. al.,2015, (School of pharmacy, R K University, Rajkot, India, 1Atmiya Institute of pharmacy, Rajkot, India)developed polymeric nanoparticles of Exemestane, for breast cancer. To achieve this goal Exemestane loaded poly (ε-caprolactone) (PCL) nanoparticles were prepared by Nanoprecipitation and Spontaneous emulsification solvent diffusion method. Nanoparticles prepared by both methods were characterized in terms of particle size, surface morphology, scanning electron microscope (SEM) and Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry (DSC). Exemestane loading was analysed by high performance liquid chromatography. In vitro drug release studies were performed in phosphate buffer saline (PBS, pH 7.4 at 37° C). The cytotoxicity was evaluated using MCF7 breast cancer cell line and Vero cell line using MTT assays. FTIR and DSC studies demonstrated no interaction between drug and polymer. Among the above two method nanoparticles prepared by Nano precipitation method resulted more effective than prepared by Spontaneous emulsification

solvent diffusion method to achieve small size (37.84 nm), uniform distribution, more recovery, high encapsulation efficiency(63.86%), higher percentage of drug release with time and more % Cell growth inhibition [11].

Meenakshi B. et.al, 2018, (Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India) improves the release rate of curcumin by microsponges prepared through quasi-emulsion solvent diffusion technique using ethyl cellulose and PVA as carriers. The micro sponges were characterized by FTIR, DSC, XRD and SEM studies followed by determination of total drug content and entrapment efficiency. The prepared microsponges were further filled in hard gelatin capsule shell and then loaded in carbopol gel to evaluate its potential in oral and topical drug delivery. Further, it was observed from the studies on release rate that microsponges filled in hard gelatin capsule shells (batch MS4) showed 93.2% release of curcumin whereas pure curcumin filled in capsule showed only 11.7% release in 8 h study. Furthermore, the microsponges loaded in carbopol gel were evaluated for ex vivo drug deposition studies and it was found that 77.5% of the curcumin was released within 24 h. The estimated drug remained in the skin was 207.61±5.03 µg/ cm^2 as determined by a Franz diffusion cell. The drug release profile data were found to be fitted best into the zero-order model with anomalous transport mechanism of drug release in both cases [12].

Bhuvaneshwaran A.et.al.,2022 (Department of Pharmaceutics, C. L. Baid Mehta College of Pharmacy, OMR, Thoraipakkam, Chennai, Tamil Nadu, India) unsaturated polyester resin and peroxide were used to fabricate microneedle mould by using 3M microneedle. Dissolving microneedle arrays transdermal patch was prepared by using 20 % w/v polymers of PVA and PVP (polyvinyl alcohol and polyvinyl pyrrolidone) and active drug 20 mg (IU) low molecular weight heparin (LMWH). The physicochemical compatibility of the drug and the polymers was studied by infrared spectroscopy suggested the absence of any incompatibility. Formulated transdermal films were physically evaluated with regard to weight variation, drug content, folding endurance, percentage of moisture content and water uptake. All prepared formulations indicated good physical stability. *In-vitro* permeation studies of formulations were performed by using Franz diffusion cells. The formulation prepared with PVP: PVA 1:2 showed best ex-vivo skin permeation through rat skin (Wistar rat) as compared to all other formulations. The release profile of the optimized formulation F2 (R^2 = 0.9933 for Higuchi) indicated that a diffusion mechanism governed the permeation of the drug from the patches. Microneedle transdermal patches were successfully prepared for LMWH, and their evaluation suggested it can have potential applications in the therapeutic arena, offering

advantages in terms of effective non-invasive delivery with improved patient compliance and bioavailability [13].

Mane P. et.al.,2022 , (University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India) Inclusion complexes of Exemestane with β-cyclodextrin and ternary agent were prepared by kneading and lyophilization technique. Different hydrophilic polymers and organic acids were screened for their influential ability as a co-complex with a carrier; β-cyclodextrin. The validation of complex formation was carried out by various solid-state techniques. The geometry of Exemestane in the β-cyclodextrin cavity was picturized in docking studies. The in vitro anticancer activity of prepared inclusion complex formulations carried out using MCF-7 cell lines [14].

6 International Status

As per WHO report "In 2020, there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer. There are more lost disability-adjusted life years (DALYs) by women to breast cancer globally than any other type of cancer. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life".

Currently, conventional therapies are used to treat breast cancer, but they have numerous shortcomings such as low bioavailability, short circulation time, and off-target toxicity. To address these challenges, nanomedicines are preferred and are being extensively investigated for breast cancer treatment. Nanomedicines are novel drug delivery systems that can improve drug stability, aqueous solubility, blood circulation time, controlled release, and targeted delivery at the tumoral site and enhance therapeutic safety and effectiveness. Nanoparticles (NPs) can be administered through different routes. Although the injectable route is less preferred than the oral route for drug administration, it has its advantages: it helps tailor drugs with targeted moiety, boosts payload, avoids first-pass metabolism, and improves the pharmacokinetic parameters of the active pharmaceutical ingredients. Targeted delivery of nanomedicine, closer to organelles such as the mitochondria and nuclei in breast cancer, reduces the dosage requirements and the toxic effects of chemotherapeutics.

Nevine S. et. al., 2012, (Department of pharmaceutics and industrial pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.) prepared and examined topical microsponge-based delivery system containing fluconazole, synthetic triazole antifungal, for controlled

release of the drug and consequently avoiding its oral effects. Ethyl cellulose & Eudragit. RS 100 based microsponges were prepared using quasi-emulsion solvent diffusion method. The effect of formulation variables such as drug to polymer ratio, polymer type, polyvinyl alcohol (PVA) concentration and type of internal phase on the physical characteristics of the microsponges was examined using 24 factorial designs. Results revealed that fluconazole loading and microsponge particle size were increased with increasing the polymer fraction. Moreover, ethyl cellulose significantly increased the drug entrapment efficiency (EE %) and the mean particle size. There was a reverse proportionality between the PVA concentration and both the EE% and the mean particle size. Regarding the solvent type, ethanol significantly increased the EE% and the particle size when compared to methylene chloride. Optimized formulation of microsponge incorporated in carbopol gel base and evaluated for its in vitro release characteristics. The developed microsponges were spherical and porous. There was no interaction between drug and polymer molecules. The drug release through cellulose dialysis membrane showed fickian release pattern. Further antifungal activity and in vivo animal studies was carried out [15].

Mukerjee, Collins, Isseroff, & Smith., 2004, (MicroInstruments & Systems Laboratory (MISL), University of California, Davis, 1 Shields Ave, Davis, CA 95616, USA) design, fabrication and testing of a hollow microneedle array with integrated, fluidic microchannels. The microneedles are machined from single crystal silicon to a shank height of 250–350m with 300m centre-to-centre spacing. The needle size, density and shape are controlled by independent processing steps. Piercing of the protective stratum corenum layer of the skin by the microneedles, providing access to the living epithelial layers underneath, is successfully demonstrated. Filling of the integrated microchannels by capillary action with both non-biological fluids (glycerol, ethanol, surrogate interstitial fluid (ISF) and water) as well as biological fluids (ISF and whole blood) is demonstrated. The ability to extract interstitial fluid from human skin is successfully achieved and verified by the in situ measurement of glucose concentration. The microchip presented here represents the first step towards the realization of a transdermal, ISF extraction and analysis microsystem. The intricate tip profile of volcano-like, a micro-hypodermic, and snake-fang type microneedle is investigated [16].

M. Y. Kim, Jung, & Park., 2012,(a Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea) Microneedle separation into the skin was mediated by hydrogel swelling in response to contact with body fluid after the needles were inserted into the skin. The hydrogel microparticles were synthesized by an emulsification method using poly-N-

isopropylacrylamide (PNIPAAm). The microneedles were fabricated by micromolding poly-lactic-co-glycolic acid (PLGA) after filling the cavities of the mold with the hydrogel microparticles. The failure of microneedle tips caused by hydrogel swelling was studied in regard to contact with water, insertion of microneedles into porcine cadaver skin *in vitro*, stress–strain behavior, and insertion into the back skin of a hairless mouse *in vivo*. The drug delivery property of the hydrogel particles was investigated qualitatively by inserting polymer microneedles into porcine cadaver skin *in vitro*, and the sustained release property of PLGA microneedles containing hydrogel microparticles was studied quantitatively using the Franz cell model. The hydrogel particles absorbed water quickly, resulting in the cracking of the microneedles due to the difference in volume expansion between the needle matrix polymer and the hydrogel particles. The swollen particles caused the microneedles to totally breakdown, leaving the microneedle tips in the porcine cadaver skin *in vitro* and in the hairless mouse skin *in vivo*. Model drugs encapsulated in biodegradable polymer microneedles and hydrogel microparticles were successfully delivered by releasing microneedles into the skin [17].

Liu et al., 2014, (Department of Neurology, Xinhua Hospital affiliated to the Medical School of Shanghai Jiao Tong University, Shanghai, China) develop novel dissolving microneedle arrays fabricated from hyaluronic acid (HA) as a material and to improve the transdermal permeability of relatively high molecular weight drugs. In this study, fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4 kDa (FD4) was used as a model drug with a relatively high molecular weight. The microneedle arrays significantly increased transepidermal water loss (TEWL) and reduced transcutaneous electrical resistance (TER), indicating that they could puncture the skin and create drug permeation pathways successfully. Both TEWL and TER almost recovered to baseline levels in the microneedle array group, and relatively small pathways created by the microneedles rapidly recovered as compared with those created by a tape stripping treatment. These findings confirmed that the microneedle arrays were quite safe. Furthermore, we found that the transdermal permeability of FD4 using the microneedle arrays was much higher than that of the FD4 solution. Furthermore, we found that the microneedle arrays were much more effective for increasing the amount of FD4 accumulated in the skin [18].

Musa MN., et al., BioImpacts, 2017, (PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Bandar Seri Begawan BE 1410, Brunei Darussalam) liquid crystalline (LC) gel formulations was prepared to deliver the anti-cancer drug exemestane through transdermal route. Two LC gel formulations were prepared by phase separation coacervation

method using glyceryl monooleate(GMO), Tween 80 and Pluronic® F127 (F127). The formulations were characterized with regard to encapsulation efficiency (EE), vesicle size, Fourier transform infrared (FTIR) spectroscopy, surface morphology (using light and fluorescence microscopy),in vitro release, ex vivo permeation, in vitro effectiveness test onMDA-MB231 cancer cell lines and histopathological analysis [19].

He J, Zhang Z, et .al .,2021,(Key Laboratory of Smart Drug Delivery of MOE, School of Pharmacy, Fudan University, Shanghai 201203, China)propranolol hydrochloride-loaded dissolving Microneedles were fabricated by using hyaluronic acid and polyvinyl pyrrolidone as matrix materials. Controllable drug loading in needle tips was achieved by a two-step casting procedure. The needles were good in mechanical strength for penetrating the SC while presented excellent dissolving capability for releasing propranolol hydrochloride. In comparison with the solution counterpart, irrespective of being applied to intact skin or solid Microneedles -pretreated skin, dissolving Microneedlessignificantly increased the permeability and skin retention of propranolol. In conclusion, dissolving Microneedles could be a potential approach for enhancing dermal delivery of propranolol to treat IH [20].

7 **Study Hypotheses:** Developsite-specific drug delivery system to increase the penetration, avoiding its first pass metabolism and decreasing its toxicity and adverse reactionby delivering drug to the site of action.

8 **Study Rationale:** Drug belongs to BCS class IV hence an increased bioavailability is desirable. The drug delivery system should be able to avoid its first-pass metabolism thereby increasing bioavailability. A transdermal site-specific drug delivery system would serve as a novel approach to deliver the drug exemestane.

9 Research Methodology

A. Characterization of drug and excipients

i. Spectroscopic studies

- Determination of absorption maxima
- Analytical method development &validation as per ICH guideline for drug estimation (UV-Vis Spectrophotometer for routine analysis and HPLC as a stability indicating method).

ii. Identification of drug by FTIR, DSC

- Authentication of drug will be carried out by using FTIR, DSC and Melting point determination.

iii. Solubility studies

- The mechanism of drug release depends on whether the drug molecules are dissolved or suspended in the delivery system and the interfacial partition coefficient of drug from delivery systems to the skin.

iv. Melting point

- The melting point will be determined by using melting point apparatus or by using DSC.

v. Crystallinity studies

- Effect of polymerization on crystallinity of the drug can be studied by powder X-ray diffraction (XRD) and Differential Scanning Colorimetry (DSC)

B. Compatibility studies:

- Drug excipient compatibility studies will be performed by using FTIR, DSC & XRD.

C. Formulation development

- There are two methods for preparation of microsponges:

1. Free Radical Suspension Polymerization (Bottom up approach): This is Bottom-up approach starting with monomer. Microsponges are prepared by free radical suspension polymerization in an emulsified liquid-liquid system. Particles forming polymerization mixtures are usually two-phase systems. In addition to the monomers and polymerization medium, another liquid (miscible with the monomer and immiscible with the medium) may also be added to the monomer to form a pore network. This liquid is known as 'monomer diluent' or 'porogen' and belongs to the category of inert, nonpolar organic solvents when added to the polymerization reaction, polymeric beads with open, porous structures can be obtained and they look just like sponges under SEM, hence the name 'microsponges'. For preparing Microsponge, the requirements are monomer namely Styrene, PHEMA, Cross linking agents are Divinyl Benzene and Porogen. It is important to maintain the

temperature for most efficient operation. Temperature of the reaction mix dictates the rate of decomposition, the initiation into free radicals and hence affecting the rate of polymerization. Once the suspension is established with discrete droplets of the desired size, polymerization is affected by activating the monomers either by catalysis, increased temperature or irradiation.

2 Quasi-emulsion solvent diffusion methods (Top down approach) this is top-down approach starting with preformed polymer. This process involves formation of quasi-emulsion of two different phases' i.e. internal phase and external phase similar to emulsions. The internal phase of drug-polymer solution made in a volatile solvent like ethanol or acetone or dichloromethane added to external phase comprising the aqueous polyvinyl alcohol (PVA) solution with vigorous stirring. Triethylcitrate (TEC), which added in an adequate amount in order to facilitate plasticity. Stirring lead to the formation of discrete emulsion globules called quasi emulsion globules. Solvent is then extracted out from these globules to form insoluble, rigid microparticles i.e. microsponges. Following sufficient stirring, the mixture is then filtered to separate the microsponges. The microsponges are then dried in an air heated oven.

Preparation of Microneedles:

1. Micromolding is the most common strategy for the fabrication of Microneedles (MNs).The MNs are fabricated by a micromolding method using hyaluronic acid (HA) and PVP-K90 via a two-step casting procedure. Briefly, the tip solution prepared by dissolving HA and PVP-K90 with a weight ratio of 1:1 in distilled water to a concentration of 30% (*w/w*). The backing solution is prepared by PVP-K90 with the concentration of 30% (*w/w*). The tip solution of 200 μL poured into the Polydimethylsiloxane (PDMS) mold, which centrifuged at 4000 rpm for 5 min to depress the solution into the PDMS mold cavities. After removing the excess solution, the PDMS mold is kept in the silica gel desiccator overnight to facilitate drying. Subsequently, 400 μL backing solution is added into the PDMS mold and then centrifuged at 4000 rpm for 10 min. The molds put in the desiccator and dried for 24 h. Finally, the dissolving MNs are gently removed from the molds and preserved in the desiccator. In this experiment, PDMS molds can be reused in the MN production process to improve fabrication efficiency.

2. The required quantity of distilled water taken and the temperature of 80 to 900 °C maintained in a magnetic stirrer. To this PVA added, and stirring continued. Once the PVA dissolved, the required quantity of PVP added. The solution kept at room temperature. From the prepared polymer solution, 6 ml taken and to this 20 mg of low molecular weight, drug added and mixed well. The mixture then added drop wise into the mould cavities and kept overnight at room temperature for drying.

- Optimization of drug-loaded microsponges/ microneedles for the breast cancer activity applying screening and optimization designs using DoE software.

D. Characterization of drug-loaded Microsponges/Microneedles

- **Particle size (Microsponges)**: Particle size and size distribution are evaluated using either an optical microscope or an electron microscope.
- **Morphology and surface topography of microsponges/microneedles**: For morphology and surface topography, various techniques can be used like photon correlation spectroscopy (PCS), Scanning electron microscopy (SEM), transmission electron microscopy (TEM) etc.
- Percent practical yield, percent drug entrapment efficiency, percent drug content is important for the development of drug delivery systems.
- The surface area and pore size of the microsponges/ microneedleshave a significant influence on the drug loading capacities.
- In vitro drug release: A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important.

E. Drug release & anti-cancer activity:

- **Franz-diffusion study**: In-vitro drug release studies will be performed by using a Franz diffusion cell with a receptor compartment capacity of 60 ml. The cellulose acetate membrane is used for the determination of the drug from the prepared transdermal patches. The cellulose acetate membrane having a pore size 0.45 μ will be mounted between the donor and receptor compartment of the diffusion cell. The prepared transdermal film will be placed on the cellulose acetate membrane and covered with aluminum foil. The receptor compartments of the diffusion cell fill with

phosphate buffer pH 7.4. The whole assembly will be fixed on a hot plate magnetic stirrer, and the solution in the receptor compartment will be constantly and continuously stirred using magnetic beads, and the temperature maintained at 32 ± 0.5 °C, because the normal skin temperature of human is 32 °C. The samples were withdrawn at different time intervals to analyze drug content spectrophotometrically. The receptor phase will be replenished with an equal volume of phosphate buffer at each sample withdrawal.

- ***Ex-vivo* Permeation Study**: An ex-vivo permeation study will be carried out by using Franz diffusion cell. Full-thickness abdominal skin of Wistar rat weighing 200 to 250 g will be used. Hair from the abdominal region to be remove carefully by using an electric clipper; the dermal side of the skin thoroughly cleaned with distilled water to remove any adhering tissues or blood vessels, equilibrate for an hour in phosphate buffer pH 7.4 before starting the experiment, and Franz diffusion cell place on a magnetic stirrer with a small magnetic needle for uniform distribution of the diffusant. The temperature of the cell is maintained at 32 ± 0.5 °C using a thermostatically controlled heater. The isolated rat skin piece mounted between the compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. A sample volume of 5 ml is removed from the receptor compartment at regular intervals, and an equal volume of fresh medium replaced. Samples filtered through Whatman filter and analyzed using spectrophotometer.

- Cell line studies for anti-cancer activity:

    i. MCF7 cells are useful for *in vitro* breast studies because they retained several ideal characteristics particular to mammary epithelium, such as the processing of estrogen, in the form of estradiol, via estrogen receptors (ER) in the cell cytoplasm. It is the first hormone responding breast cancer cell line. Its unique properties are useful for experimental therapeutics. They are also sensitive to cytokeratin. When grown *in vitro*, the cells are capable of forming domes and the epithelial-like cells grow in monolayers.

    ii. T47D is another cell line that is often used to represent luminal A breast cancer as it is ER+ and hormone-dependent.

    iii. HCC-1428 and MDA-MB-361 endocrine-resistant models

- **In-vivo studies**: The efficacy of site-specific anticancer drug delivery determined by administering the formulation to Kunming mice implanted with 4T1 cells for tumor induction. After seven days of tumor inoculation, when the tumor volume approached 100–200 mm^3, the treatments began, and that day mark as day 1. Each group of mice randomly assigned to one of four treatment groups with four mice in each group, and each group of mice treated twice weekly for three weeks of various formulations. Tumor volume development, survival time, and body weight changes in mice were checked after treatment.

F. Stability studies.

- Stability studies will be performed according to the ICH guidelines. The prepared formulation will be tested for stability on storing them at required temperature and humidity .After specified duration of time they will be evaluated for the following parameters like appearance, pH, drug content analysis, drug release profiles, Rheological properties etc. HPLC will be performed for stability sample.
- Stability studies of the optimized formulation will be carried out by storing the replicates as per ICH guidelines for 6 months. Samples will be withdrawn every two weeks to measure stability indicating parameters.

10 Statistical Plan

Sr. No.	Expected target to be achieved	1-6 months	7-12 months	13-18 months	19-24 months	25-30 months	31-36 months
1	Literature survey						
2	Preformulation studies						
3	Analytical method development						
4	Formulation development and optimization						
5	Evaluation of optimized drug delivery systems						
6	In-vitro/Ex-vivo study						
7	Cell-line / animal study						
8	Statistical Analysis, Data Interpretation and Conclusions						

	Publishing Research and submitting data						

11 Limitations of the Study

The site-specific drug delivery systems are not much explored for the above-mentioned drug the tunable particle size studies related to improved drug delivery of the anti-cancer drug will be challenging. Few of the manufacturing and characterization studies of the site-specific drug delivery systems need specialized equipment.

12 Expected Outcomes of Study

The expected outcomes of the proposed research study are as follows

- Formulation of an efficient site-specific drug delivery system (microsponges/ microneedles).
- Developing an optimized and stable formulation for treatment of breast cancer.
- Formulation of a carrier system which will overcome the drawbacks of conventional anti-cancer formulations available currently.

13 Select Bibliography

1. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, Ji X, Liu W, Huang B, Luo W, Liu B, Lei Y, Du S, Vuppalapati A, Luu HH, Haydon RC, He TC, Ren G. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018 May 12; 5(2):77-106. Doi: 10.1016/j.gendis.2018.05.001. PMID: 30258937; PMCID: PMC6147049.
2. Alkabban FM, Ferguson T. Breast Cancer. 2022 Sep 26. In: StatPearls. Treasure Island (FL): Stat Pearls Publishing; 2022 Jan–. PMID: 29493913.
3. Schiff R, Chamness GC, Brown PH. Advances in breast cancer treatment and prevention: preclinical studies on aromatase inhibitors and new selective estrogen receptor modulators (SERMs). Breast Cancer Res. 2003; 5(5):228-31. Doi: 10.1186/bcr626. Epub 2003 Jul 28. PMID: 12927029; PMCID: PMC314430.
4. Fan W, Chang J, Fu P. Endocrine therapy resistance in breast cancer: current status,

possible mechanisms and overcoming strategies. Future Med Chem. 2015 Aug; 7(12):1511-9. Doi: 10.4155/fmc.15.93. Epub 2015 Aug 26. PMID: 26306654; PMCID: PMC5558537.

5. Chumsri S, Howes T, Bao T, Sabnis G, Brodie A. Aromatase, aromatase inhibitors, and breast cancer. J Steroid Biochem Mol Biol. 2011 May; 125(1-2):13-22. doi: 10.1016/j.jsbmb.2011.02.001. Epub 2011 Feb 16. PMID: 21335088; PMCID: PMC3104073.
6. Amaral C, Lopes A, Varela CL, da Silva ET, Roleira FM, Correia-da-Silva G, Teixeira N. Exemestane metabolites suppress growth of estrogen receptor-positive breast cancer cells by inducing apoptosis and autophagy: A comparative study with Exemestane. Int J Biochem Cell Biol. 2015 Dec; 69:183-95. doi: 10.1016/j.biocel.2015.10.024. Epub 2015 Oct 26. PMID: 26515125.
7. Basu P, Maier C. Phytoestrogens and breast cancer: In vitro anticancer activities of isoflavones, lignans, coumestans, stilbenes and their analogs and derivatives. Biomed Pharmacother. 2018 Nov; 107:1648-1666. doi: 10.1016/j.biopha.2018.08.100. Epub 2018 Sep 8. PMID: 30257383.
8. Basu P, Maier C. Phytoestrogens and breast cancer: In vitro anticancer activities of isoflavones, lignans, coumestans, stilbenes and their analogs and derivatives. Biomed Pharmacother. 2018 Nov; 107:1648-1666. doi: 10.1016/j.biopha.2018.08.100. Epub 2018 Sep 8. PMID: 30257383.
9. Shashank Chaturvedi, Anuj Garg,A comprehensive review on novel delivery approaches for exemestane, Journal of Drug Delivery Science and Technology, Volume75,2022,103655,ISSN1773-2247,https://doi.org/10.1016/j.jddst.2022.103655.
10. Eric Scholar, Exemestane, Editor(s): S.J. Enna, David B. Bylund, xPharm: The Comprehensive Pharmacology Reference, Elsevier, 2007, Pages 1-4, ISBN 9780080552323,https://doi.org/10.1016/B978-008055232-3.62977-0.
11. Kanani, M. & Vadalia, K. (2015). Formulation, evaluation and comparison of exemestane nanoparticles prepared by nanoprecipitation method and spontaneous emulsification solvent diffusion method. 8. 236-249.
12. Bhatia M, Saini M. Formulation and evaluation of curcumin microsponges for oral and topical drug delivery. Prog Biomater. 2018 Sep; 7(3):239-248. doi: 10.1007/s40204-018-0099-9. Epub 2018 Sep 21. PMID: 30242738; PMCID: PMC6173672.
13. Avcil, M.; Çelik, A. Microneedles in Drug Delivery: Progress and

Challenges. *Micromachines* **2021**, *12*, 1321. https://doi.org/10.3390/mi12111321.

14. Mane, p. T.; wakure, b. S.; wakte, p. S. Encapsulation of exemestane with β-cyclodextrin and ternary agent: formulation, evaluation and anticancer activity. *Int J App Pharm* **2022**, *14*, 119-127.
15. Nevine SA, Shahira FEM. A new topical fluconazole microsponge loaded hydrogel: preparation and characterization. Int. J.Pharm. Pharm. Sci. 2012; 4(1): 460-468.
16. E.V. Mukerjee, S.D. Collins, R.R. Isseroff, R.L. Smith, Microneedle array for transdermal biological fluid extraction and in situ analysis, Sensors and Actuators A: Physical, 114 (2–3), **2004**, 267-275, ISSN 0924-4247, https://doi.org/10.1016/j.sna.2003.11.008.
17. Hong, X., Wu, Z., Chen, L. *et al.* Hydrogel Microneedle Arrays for Transdermal Drug Delivery. *Nano-Micro Lett.* **6**, 191–199, **2014**. https://doi.org/10.1007/BF03353783.
18. Shu Liu, Mei-na Jin, Ying-shu Quan, Fumio Kamiyama, Kosuke Kusamori, Hidemasa Katsumi, Toshiyasu Sakane, Akira Yamamoto, Transdermal delivery of relatively high molecular weight drugs using novel self-dissolving microneedle arrays fabricated from hyaluronic acid and their characteristics and safety after application to the skin, European Journal of Pharmaceutics and Biopharmaceutics, 86 (2),**2014**, 267-276, ISSN 0939-6411, https://doi.org/10.1016/j.ejpb.2013.10.001.
19. Musa MN, David SR, Zulkipli IN, Mahadi AH, Chakravarthi S, Rajabalaya R. Development and evaluation of exemestane-loaded lyotropic liquid crystalline gel formulations. Bioimpacts. 2017; 7(4):227-239. doi: 10.15171/bi.2017.27. Epub 2017 Sep 3. PMID: 29435430; PMCID: PMC5801534.
20. He, J.; Zhang, Z.; Zheng, X.; Li, L.; Qi, J.; Wu, W.; Lu, Y. Design and Evaluation of Dissolving Microneedles for Enhanced Dermal Delivery of Propranolol Hydrochloride. *Pharmaceutics* **2021**, *13*,579.https://doi.org/10.3390/pharmaceutics13040579
21. Opatha, S.A.T.; Titapiwatanakun, V.; Boonpisutiinant, K.; Chutoprapat, R. Preparation, Characterization and Permeation Study of Topical Gel Loaded with Transfersomes Containing Asiatic Acid. *Molecules* **2022**, *27*, 4865. https://doi.org/10.3390/molecules27154865.
22. Sh. Ahmed, A.; Taher, M.; Mandal, U. K.; Jaffri, J. M.; Susanti, D.; Mahmood, S.; Zakaria, Z. A. Pharmacological Properties of Centella Asiatica Hydrogel in Accelerating Wound Healing in Rabbits. *BMC Complement. Altern. Med.***2019**, *19* (1), 213. https://doi.org/10.1186/s12906-019-2625-2.

23. Jain, P. K.; Agrawal, R. K. High Performance Liquid Chromatographic Analysis of Asiaticoside in Centella Asiatica (L.) Urban. *Chiang Mai J Sci.*
24. Kwon, K. J.; Bae, S.; Kim, K.; An, I. S.; Ahn, K. J.; An, S.; Cha, H. J. Asiaticoside, a Component of Centella Asiatica, Inhibits Melanogenesis in B16F10 Mouse Melanoma. *Mol. Med. Rep.***2014**, *10* (1), 503–507. https://doi.org/10.3892/mmr.2014.2159.
25. Biswas B, Biswas G, Ganguly S, Ghosh J, Roy S, Roy MK, Pipara A, Karmakar J, Mukherjee N, Chakraborty S, Mishra DK, Midha D, Dabkara D. Not so 'rare'-an example of malignant melanoma in India: report from a tertiary cancer centre. E cancer medical science. 2021 Dec 16; 15:1335. doi: 10.3332/ecancer.2021.1335. PMID: 35211204; PMCID: PMC8816508.
26. Rajan K. Verma , Sanjay Garg. Current Status of Drug Delivery Technologies and Future Directions. Pharmaceutical Technology On-Line. 2001; 25 (2): 1–14.
27. S. Madhav, A. Saini. Niosomes: A novel drug delivery system. International Journal of Research in Pharmacy and Chemistry, 2011; 1: 498-511.
28. D.Breimer, R.Speiser. Topics in pharmaceutical sciences. Elsevier Science Publishers, New York, USA. 1985; 291.
29. NunoMartinho, Christiane Damgé, Catarina Pinto Reis. Recent Advances in Drug Delivery Systems. Journal of Biomaterials and Nan biotechnology, 2011; 2: 510-526.
30. O. Katare, K. Raza, B. Singh, S. Dogra. Novel drug delivery systems in topical treatment of psoriasis: Rigors and vigors. Indian Journal of Dermatology,Venereology and Leprology, 2010; 76: 612-621.
31. He J, Zhang Z, Zheng X, Li L, Qi J, Wu W, Lu Y. Design and Evaluation of Dissolving Microneedles for Enhanced Dermal Delivery of Propranolol Hydrochloride. Pharmaceutics. 2021 Apr 19; 13(4):579. doi: 10.3390/pharmaceutics13040579. PMID: 33921712; PMCID: PMC8072810.